

# Desensitization by covalent modification of the chemoreceptor of *Escherichia coli*

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Received 8 January 1986

Chemoreceptors in *Escherichia coli* were studied in situ in chemotactic mutants, deficient in the ability to modify the receptors, by using membrane vesicles prepared from the mutants. The affinity of the receptors for the ligands is related to the level of modification of the receptors. Unmodified serine receptor had a dissociation constant of 0.8  $\mu\text{M}$ , while modified receptor had a dissociation constant that was at least 100-times higher. The results are discussed in relation to the two-state model of the chemoreceptor.

*Chemotaxis    Desensitization    Protein methylation    Bacterial chemoreceptor    (E. coli)*

## 1. INTRODUCTION

A set of methylatable transmembrane receptors mediates the processing of chemotactic signals in gram-negative bacteria such as *Escherichia coli* and *Salmonella typhimurium*. It is believed that the receptors accept chemicals on the domain that faces the outside of the cell and that the receptors are reversibly methylated on the domain that faces the inside of the cell. Methylation and demethylation of the receptor are indispensable for successful chemotaxis since a mutant lacking the ability to perform either reaction is not chemotactic. The chemotactic machinery involving the receptor controls the direction of turn of the flagellar motor which is attached to a helical screw of the flagellar filament. The cells swim smoothly or tumble, depending on the direction of revolution (review [1]). A mutant lacking the ability to methylate the receptor swims smoothly; contains non-modified receptors; and never adapts to chemical stimuli [2–5]. A mutant lacking the ability to demethylate receptor tumbles; responds only to very high concentrations of attractants; contains modified receptors; and never adapts [6–10]. The two-state model of the chemoreceptor was formulated to explain all of the characteristics mentioned above [11].

In this study we designed an experiment to examine possible desensitization of the receptor as a result of methylation. To avoid perturbations from the methylation-demethylation system, we used mutants that lack either methyltransferase or methylesterase or both. The results showed that desensitization depends on chemical modification of the receptor.

## 2. MATERIALS AND METHODS

### 2.1. Supplies

Centrifree, an ultrafiltration device, was purchased from Amicon (MA, USA). Radioactive amino acids L-[U- $^{14}\text{C}$ ]serine and L-[ $^3\text{H}$ ]aspartate were obtained from New England Nuclear, and their specific activities were 173 mCi/mmol and 8 Ci/mmol, respectively. The specific activities of these amino acids were adjusted by adding non-radioactive species when necessary.

### 2.2. Bacterial strains

All chemotactic mutants of *E. coli* were derived and supplied to us by J.S. Parkinson of the University of Utah [8,10,12]. They are summarized in table 1. The cells were cultured at 30°C in a medium that contained 0.5% NaCl, 0.5% bacto-tryptone, 0.3% yeast extract and 0.1% maltose.

Table 1  
Bacterial strains used in this study

Strain	Phenotype	Genotype
W3110	wild type	
RP4080	<i>cheR</i>	F <sup>-</sup> <i>thi thr leu his</i> $\Delta(gal-att\lambda)$ <i>rpsL recA cheR217</i>
RP1061	<i>cheR</i>	F <sup>-</sup> <i>thi leu his rpsL</i> $\Delta(tap-cheB)2241$ ( $\lambda cheB^+$ )
RP2867	<i>cheR-cheB</i>	F <sup>-</sup> <i>thi thr leu his metF strA cheRB2241</i>
RP477	<i>cheB</i>	F <sup>-</sup> <i>thi thr leu his</i> $\Delta(gal-att\lambda)$ <i>strA cheB294(am)</i>
RP4953	<i>cheB</i>	F <sup>-</sup> <i>thi his pyrC thyA araD lacU169 gyrA rpsL</i> $\Delta(cheB)m62-16$

Cells in late logarithmic growth phase were harvested and membrane vesicles were prepared according to published methods [7,13].

### 2.3. Binding assay

Binding of serine and aspartate to their cognate receptor was measured by the isotope displacement method described by Clark and Koshland [13] except that free amino acids in the assay mixture were removed by ultrafiltration instead of ultracentrifugation. Radioactivity was determined in an Aloka liquid scintillation counter with an external standard to correct for counting efficiency. Use of ultrafiltration made the rapid isolation (3 min) of free ligands possible, and suppressed any possible artifacts that could result from incorporation of ligands into the vesicles. The assay mixture contained 16 mg of membrane protein, and the total volume was 800  $\mu$ l for each sample. The amounts of free ligand and free plus bound ligand were determined for each assay by dividing the assay mixture into two portions. A portion of 100  $\mu$ l of solution containing ligand was counted.

## 3. RESULTS AND DISCUSSION

### 3.1. Binding of serine

It has been established that binding of serine and aspartate is dependent on the presence of separate and different chemoreceptors and that the serine receptor comprises more than half of the total population of methylatable chemoreceptors [7,13,14]. We measured the binding of serine to membrane vesicles prepared from chemotactic mutants. The receptor in vesicles derived from the *cheR* mutant (methylation deficient) showed a typical saturation curve, with a half-maximum

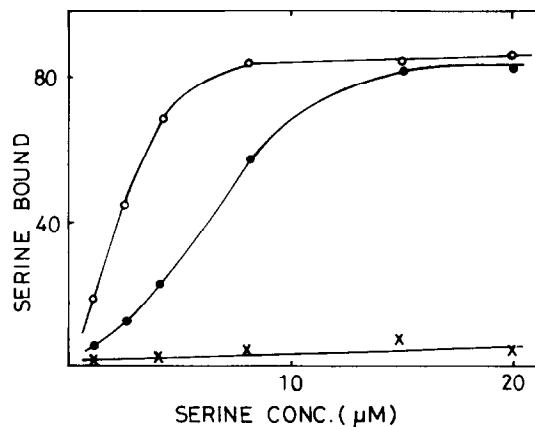


Fig.1. Binding of serine to membrane vesicles. The assay was done as described in section 2. The units on the vertical axis are pmol serine bound per mg membrane protein. (○) Receptor in *cheR*, (●) receptor in *cheR-cheB*, (×) receptor in *cheB*.

binding at a serine concentration of  $8 \times 10^{-7}$  M. The receptor in vesicles from a *cheR-cheB* mutant (methylation and demethylation deficient) showed a slightly higher concentration for half-maximum binding. However, the receptor from a *cheB* mutant (demethylation deficient) showed less than 10% of the binding expected from other experiments. The amount of receptor protein in membrane vesicles was not affected by the mutations as determined by two-dimensional gel electrophoresis (not shown – similar results in [5,7]). Two different strains of *cheB* mutants, one being a deletion mutant and the other an amber mutant, yielded essentially the same result, and we concluded that the receptor in *cheB* mutants has a lower apparent affinity for the ligand. This conclusion is consistent with many behavioral studies that

demonstrate a high threshold to serine in *cheB* mutants [8,9]. Behavioral studies also showed so-called potentiation [13]. However, addition of other attractants, for example aspartate, did not affect the affinity for serine within the range of concentrations employed in the present study. The present assay system contained serine receptor in a concentration range around  $10^{-6}$  M. Hence, very little binding would be detected when the dissociation constant was larger than  $10^{-4}$  M. Even though we could not determine an exact binding constant for serine for the receptor in the *cheB* background, we believe the evidence is sufficient to show desensitization of the receptor by methylation and amidation, since some of the methylatable sites are synthesized in amide form and the amide group is removed by the *cheB* function [10,14]. Here we have not distinguished between the effects of methylation and amidation since it is probable that both modifications have similar, if not identical, effects on the receptor. When the receptor in wild type cells was assayed as described above, the binding of serine was not monophasic. This result is in agreement with the presumption that the receptor is heterologous in terms of its level of methylation.

### 3.2. Binding of aspartate

Binding of aspartate to its receptor showed its half-maximum level at an aspartate concentration of  $8 \times 10^{-7}$  M when assayed with vesicles derived

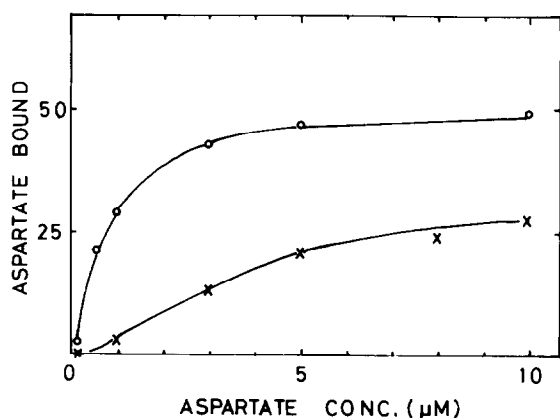
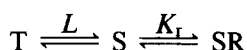


Fig.2. Binding of aspartate to membrane vesicles. The assay was done as in fig.1. The units on the vertical axis are the same as in fig.1. (○) Receptor in *cheR*, (×) receptor in *cheB*.

from a *cheR* mutant. In contrast to the case of serine receptor, desensitization of the receptor by the covalent modification was not significant. The half-maximum concentration was only about 10-times larger than in the case of unmodified receptor.

### 3.3. Relation to the two-state model

The two-state model of chemoreceptor considers two conformational states, namely the tumble-signaling state (T) and the smooth-signaling state (S). The attractant has affinity for the S-state. Thus,



Here,  $L$  and  $K_r$  denote the equilibrium constants of each step, and  $R$  represents the ligand. In the absence of ligand the receptor fluctuates between the two states and the probability of transition can be regarded as a function of the modification state: the higher the level of modification, the higher the probability of the receptor being in the T-state. Here the value of  $L$  is a constant, specific for each mutant, since no change in the level of modification takes place. The fractional occupancy of the receptor,  $p(r)$ , at a given concentration of ligand is,

$$p(r) = [R]/[R] + K_r(1 + L)$$

If we choose  $8 \times 10^{-7}$  M as the value of  $K_r$ , we have parallel sigmoidal curves for different values of  $L$ . Fig.3 shows the shape of  $p(r)$  versus  $\log[R]$  with different  $L$  values. A plot of data obtained experimentally indicates that the  $L$  value for the fully modified serine receptor is very close to 100 while that for the aspartate receptor is about 10. Differences in the degree of desensitization between the serine and aspartate receptors may be related to the difference in the number of moieties introduced into a single molecule. It is also possible that the methyl moiety and the amino moiety alter the value of  $L$  in different ways.

### 3.4. Conclusion

Bacterial chemoreceptors undergo desensitization via a system of methylation and demethylation. The level of methylation is also important within the organisms for comparison between the

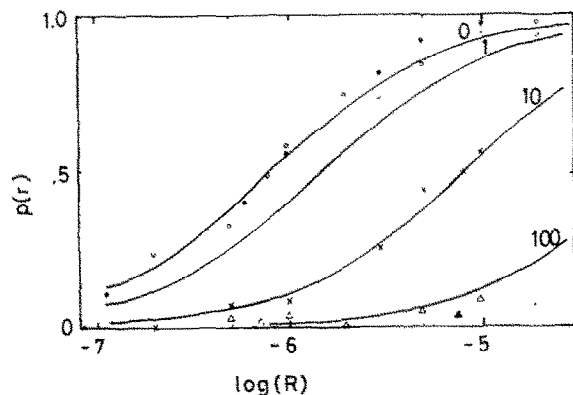


Fig.3. Plot of the experimental data and theoretical curves. Assuming the two-state model for the receptor, fractional occupancy of the receptor was calculated theoretically. Experimental data are plotted on the same graph. Solid lines are the theoretical curves for different  $L$  values, which are shown beside each curve. Each point of experimental data was obtained by averaging 3 assays (deviation less than 7%). In the case of *cheB* membrane, the assumption was made that the maximum binding is the same as for *cheR* membrane. (○) Serine binding to the receptor in *cheR*, (●) aspartate binding to the receptor in *cheR*, (×) aspartate binding to the receptor in *cheB*, (Δ) serine binding to the receptor in *cheB*.

present and the past since it functions as an internal index by which the organisms can decide the direction of response. This mechanism may be related to many chemical modifications of receptors that operate with sensation-response coupling, in a broader context. For example, a receptor for cell growth factor is known to be covalently modified.

## ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for scientific research on Molecular Mechanism of Bioelectrical Response (project no.60115001) from the Japanese Ministry of Education, Science and Culture.

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